## I. Rejections under 35 U.S.C. § 103 over Ben-Ezra in view of Shibata and McKenzie

Claims 18-36 and 42-45 stand rejected under 35 U.S.C. § 103(a) as allegedly unpatentable over Ben-Ezra et al., (*J. Histochemistry & Cytochemistry* 39: 351-54 (1991)) ("Ben-Ezra") in view of Shibata et al. (*Am. J. Pathol.* 121: 539-543 (1992)) ("Shibata") and McKenzie et al. (U. S. Patent No. 5,491,062) ("McKenzie").

The Office admits that Ben-Ezra, the primary reference, fails to disclose microscopic analysis of the specimen or centrifugation following the boiling step. Microscopic analysis is part of pathologic analysis by a pathologist and expected as part of the standard of care diagnosis provided to patients. *See* ¶¶7-10, Declaration under 37 C.F.R. § 1.132 of Dr. Sydney Finkelstein ("Declaration"). Additionally, the missing centrifugation step, is a step which unexpectedly produces a sample that can consistently and reliably amplified using PCR. *See* ¶18, Declaration. This is important when trying to improve upon accepted procedures for standards of diagnostic care. Finally, Ben-Ezra teaches that the tissue sample must be many thousand fold larger than the sample needed utilizing the claimed methods. *See*, e.g., ¶¶20-23, Declaration. As the cited reference indicates, although these steps at first blush may appear simple, they are far from obvious or anticipated based on what was known and taught in the literature at the time. *See*, e.g. ¶¶35-38, Declaration.

The secondary references of Shibata and McKenzie fail to cure the defects that are inherent to Ben-Ezra. First, Shibata teaches a methodology that is (1) economically wasteful (*e.g.,* single usage pens), leads to further damage to the DNA being sampled (*i.e.*, damage by fixation, staining, and ink), and uses ultraviolet light treatment of the tissue sample, a step which is not utilized in the claimed methods. *See* ¶¶ 24-26, Declaration. The Office cites Shibata for teaching the targeting of particular microscopic sized specimens. However, as discussed in the attached Declaration, the methods of Shibata were ones that did not lend themselves well to standardized pathology screening practices, and in fact, apparently have not been successfully implemented by anyone other than the authors themselves. *See* ¶¶24, 26, and 30, Declaration. The fact that Shibata taught use of microscopic amounts is

a step that is also used in combination with fixation, staining, and ink dotting, and UV light exposure. Additionally, Shibata is either detecting human papilloma virus, which is present in large amounts, or detection of P53 by a total 42 PCR cycles and apparently long term exposure to X-ray film after $^{32}$P-labeling. *See*, ¶29, Declaration. Such a high number of PCR cycles as well as long-term exposure to X-ray film would not be acceptable to pathology standard of diagnostic care, because it would require too much time before a diagnosis could be rendered for the individual patient.

McKenzie also fails to cure the defects inherent to both Shibata and Ben-Ezra. McKenzie is directed to the detection of mycoplasma in cell cultures and in animals. *See* Abstract. The methodology involves detection not of fixative or stained cells, but rather fresh cells. DNA quality if far greater in fresh cells than in fixative treated, stained, and/or inked cells. Despite the high quality of DNA being used, McKenzie nevertheless uses $2x10^5$ to $1x10^6$ fresh cells. This is about 3 times more than the number of cells of the presently claimed methods. Additionally, McKenzie does not suggest let alone teach that the method can be used on fixative treated and/or stained cells as well. At col. 8, line 60 to col. 9, line 11, McKenzie teaches in one example, that the method of obtaining DNA involves a proteinase K digestion for 4-16 hours, followed by a phenol-chloroform extraction and then a 20 minute centrifugation at 12,000xg. The aqueous phase was then removed, and a second extraction was performed on the DNA-containing lysate using phenol-chloroform. The aqueous phase of the second extraction was recovered and subjected to a 2 M ammonium acetate precipitation. Samples were re-suspended and diluted to "appropriate concentrations" for the PCR reactions. When dealing with minute quantities of DNA from fixative and/or stained treated samples, such methods would be ineffective. ¶¶31-34, Declaration.

In the next example at col. 9, lines 12-28, McKenzie teaches a boiling extraction of DNA. One million fresh cells were collected, counted, and washed (3 times the number used in the claimed methods). See, ¶31, Declaration. Again the cells are not subject to staining and/or fixation. Additionally, a known number of cells are used. The boiling step is performed using 1X *Taq* reaction buffer with centrifugation. The lysates obtained were again diluted to appropriate

concentrations. At col. 15, lines 19-22, McKenzie points out that the cell washing step was needed to remove components in media which interfere with Intergenic Length Polymorphism (ILP) PCR. This cannot be done with fixative and/or stained cells and there is no teaching, in the patent of how cells subjected to such treatment would be processed. *See* ¶31, Declaration. Additionally, McKenzie teaches that the purified DNA must be accurately measured. *See* McKenzie at col. 9, lines 9-11. However, measuring DNA content cannot be performed with accuracy using the claimed methods.

Additionally, McKenzie is directed to mycoplasma detection primarily of cell cultures (*i.e.*, fresh cells). Mycoplasmas are wall-less eubacteria which exist as parasites. *See* McKenzie at col. 1, lines 14-26. They will be present in multiple copies. The detection method of McKenzie hinges on detection of mycoplasma specific tRNAs, which are again present in multiple copies. *See* ¶32, Declaration. Accordingly, the methods of McKenzie cannot suggest for the claimed methods, which are not directed to bacterial tRNA detection in fresh cells (¶34, Declaration), and do not cure the deficiencies of the Shibata and Ben-Ezra methods.

As stated in the response dated March 23, 2005, the references when viewed for what they teach ***as a whole*** do not teach or suggest the claimed methods. Nor do these references alone and in combination provide motivation to pick and choose between the numerous steps present in each of the references to reach the claimed methods. The resultant combination is not obvious unless the prior art also suggests the desirability of the combination. *See In re Mills,* 916 F.2d 680, 16 U.S.P.Q.2d 1430 (Fed. Cir. 1990). Neither Ben-Ezra, Shibata, nor McKenzie alone and in combination teach the claimed combination of steps. Nor would the prior art at the time have suggested the desirability of the combination. In fact, based on what was known at the time regarding fixed tissue, stained tissue, presence of tissue in samples to be amplified by PCR, the need for extraction steps such as phenol-extraction, and sample size, there are numerous factors which Applicants have applied to the claimed method which the art at the time would have taught away from performing. *See* ¶¶6-10, Declaration.

Accordingly, if there is no motivation to combine the references and in fact a teaching away based on the art at the time, and even in the art after the priority date of the instant application, there can be no expectation of success. The Federal Circuit Court of Appeals has articulated the requirements of a proper analysis as follows:

> [W]here claimed subject matter has been rejected as obvious in view of a combination of prior art references, a proper analysis under § 103 requires, inter alia, consideration of two factors: (1) whether the prior art would have suggested to those of ordinary skill in the art that they should make the claimed composition or device, or carry out the claimed process; and (2) whether the prior art would also have revealed that in so making or carrying out, those of ordinary skill would have a reasonable expectation of success. *See In re Dow Chemical Co.*, ... 5 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1988). Both the suggestion and the reasonable expectation of success must be founded in the prior art, not in the applicant's disclosure.

*In re Vaeck*, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991). The Office Action appears to focus on the obviousness of substitutions and differences instead of on the claimed invention taken ***as a whole***. This "is a legally improper way to simplify the often difficult determination of obviousness." *The Gillette Co. v. S.C. Johnson & Sons, Inc.*, 16 U.S.P.Q.2.d 1923, 1927 (Fed. Cir. 1990), citing with approval, *Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 231 U.S.P.Q. 81 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987). In other words,

> [i]t is wrong to use the patent in suit as a guide through the maze of prior art references, combining the right references in the right way so as to achieve the result of the claims in suit.

*Orthopedic Equipment Company v. U.S.*, 217 U.S.P.Q. 193, 199 (Fed. Cir. 1983). Thus, claims 18-46 and 42-45 are nonobvious for the reasons stated above, and the rejection should respectfully be withdrawn.

## II. Rejection of Claims 37-41 over Ben-Ezra in view of Shibata, McKenzie, and Perlin under 35 U.S.C. § 103

On page 18 of the March 23 response, Applicants asserted that the combination of Ben-Ezra, Shibata, McKenzie, and Perlin (U.S. Patent No. 5,580,728) did not teach or suggest the claimed methods. Ben-Ezra, Shibata, and McKenzie all fail to teach or suggest the claimed methods, and do not provide motivation to be combined to reach the claimed methods as discussed *supra*, in the Declaration under 37 C.F.R. § 1.132 by Sydney Finkelstein, and as previously argued. The Office cites Perlin for allegedly teaching a method and a system for genotyping using a computer and a database. In fact, Perlin, as a whole, is not directed to specialized extraction of DNA from fixative and/or stained tissue, but rather converting labeled PCR products into signal, removing PCR stutter pattern, and determining the genotype of the location of the DNA. *See* Abstract, Perlin. Perlin alludes to standardized means of extracting DNA from fresh tissue that has not been subject to fixation and/or staining. *See* col. 8, lines 42-55, Perlin. Additionally, the DNA is extracted by proteinase K digestion, followed by phenol-chloroform extraction and sodium acetate precipitation of the DNA. *Id.,* at col. 8, line 55 to col. 9, line 9. Accordingly, Perlin does not address the defects identified for Ben Ezra, Shibata, and McKenzie above. Accordingly, Perlin cannot cure the defects of Ben Ezra, Shibata, and McKenzie, let alone provide motivation for the asserted combination. Therefore, Applicants respectfully request withdrawal of the rejection claims 37-41 under § 103, and respectfully requests allowance of these claims.

## III. Unexpected Results

The presence of unexpected, advantageous, or superior results is itself evidence of nonobviousness. *See, e.g.,* M.P.E.P. § 716.02(a); *In re Papesch*, 315 F.2d 381, 137 U.S.P.Q. 43 (C.C.P.A. 1963). Applicants provide in the attached Declaration and associated referenced articles evidence that pathology practice requires tissue fixation, which is frequently followed by tissue staining. *See* ¶¶ 6-19, Declaration. Both tissue fixation and staining, both individually, as well as when the

steps are combined, cause changes to the DNA which were known in the art at the time to make amplification of the DNA using PCR more difficult. Larger amounts of tissue would have therefore have been expected to be required to obtain a similar result as that obtained when using unfixed or unstained tissue. Thus, the results of using small amounts of fixative and/or stained tissue and obtaining consistently good results was unexpected.

Another unexpected feature of the claimed methods is that the centrifugation step is sufficient to produce a sample of DNA, even with tissue present, that was capable of being amplified using PCR. As discussed in ¶¶18-19 of the Declaration, having large amounts of tissue present and not having the sample further treated with a phenol- extraction step went contrary to what was known in the art at the time regarding requirements for reliably amplifying DNA from samples. In fact, even in 2000, practitioners cautioned against using crude lysates containing tissue, which would interfere with PCR. *See* ¶¶18-19, Declaration.

Therefore, the results obtained using the claimed steps were unexpected given what practitioners knew at the time, and even recently. Unexpected results are indicia of nonobviousness. Accordingly, Applicants respectfully request withdrawal of the rejections under § 103 and allowance of the claims.

## CONCLUSION

From the foregoing, further and favorable notice in the form of a Notice of Allowance is respectfully requested, and such action is earnestly solicited. In the event that there are any questions concerning this reply or the application in general, the Examiner is respectfully requested to telephone the undersigned representative so that prosecution of the application may be expedited.

If any further fees are due to maintain pendency of this application, the Office is authorized to charge such fees to Deposit Account No. 50-0573.

Respectfully submitted,

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